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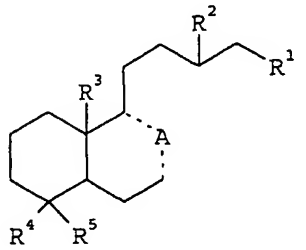
(54) Biologically active labdane or labdene derivatives from Cistus

(57) The present invention provides a biologically active composition which has particularly an inhibitory activity on production of melanin, a cell activating activity and an anti-bacterial activity, are derived from natural sources, and are safe and not harmful; one or several compounds represented by the following general formula (1) are contained in said composition:



(1)

wherein R^1 represents CH_2OH, $-COOR^6$ or -COOX, in which X is a group capable of forming a salt and R^6 represents hydrogen or a C_1 to C_3 lower alkyl group; R^2 to R^5 each represent hydrogen or a methyl group; and ---A--- represents $=C(CH_3)-$, $-C(CH_3)=$, $-C(=CH_2)-$, $-CH(CH_3)-$ or $-C(OH)(CH_3)-$.

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Description

BACKGROUND OF THE INVENTION

Field of the invention

[0001] The present invention relates to a biologically active substance consisting of specific compounds. Further, the present invention relates to a melanin production inhibitor, cell growth activator, anti-bacterial agent etc. containing the above-mentioned compounds. Further, the present invention relates to an agent for external application onto the skin, oral cavity compositions, bath additives etc.

Prior Art

[0002] Compounds with a wide variety of biological activities have been developed heretofore. In particular there are a large number of reports on compounds derived from natural materials especially from the safety point of view.

[0003] As typical compounds having biological activity, those having an inhibitory effect on production of melanin are described hereinafter.

[0004] As the age advances, stains, freckles or deposition of pigment after sunburn tends to be formed, increased or settled into the skin. This is problematic particularly for the middle-aged and the elderly.

[0005] Although there are still unrevealed aspects of the mechanism of generation of such acquired pigment (melanin) deposition, it is believed that melanin pigment formation is caused by hormone abnormality and external stimuli such as ultra violet rays from sunlight, oxygen and chemical substances etc., and abnormally deposited in the skin. The development of compounds preventing the formation and deposition of this melanin is highly desired and many compounds have been developed theretofore. These compounds include ascorbic acid and derivatives thereof, a placenta extract, hydroquinone, kojic acid, arbutin and ellagic acid, and further there are large number of reports on melanin production-inhibiting components extracted from plants, and these include a chamomile extract, namely an extract of ***Matricaria chamomilla*** L. which belongs to the ***Compositae*** (Japanese Patent Application Laid-Open No. 8- 92056), a golden flower root extract, namely an extract of ***Scutellaria baicalensis*** G. which belongs to the ***Labiatae*** (Japanese Patent Application Laid-Open No. 8-104616), a cumin seed extract, namely an extract of ***Cuminum cyminum*** L. which belongs to the ***Umbelliferae*** (Japanese Patent Application Laid-Open No. 8-119848) and a wolo extract, namely an extract of ***Borassus flabellifera*** which belongs to the ***Palmae*** (Japanese Patent Application Laid-Open No. 10-29928). Further, the present inventors also found that a fraction obtained by purifying solvent extracts from various plants by silica gel chromatography strongly inhibits the production of melanin in B16 melanoma cells, and filed for a patent (Japanese Patent Application No. 9254025, August 15, 1997). As a substance having a labdan structure, manool, an extract of ***Dacrydium biforme*** is reported to have an inhibitory effect on production of melanin (Japanese Patent Application Laid-Open No. 6-72855). Further the inhibitory effect of derivatives thereof on melanin production is also reported (Japanese Patent Application Laid-Open Nos. 725754, 7-69858, 7-206625 etc.).

[0006] However, the majority of these conventional melanin production inhibitors are inadequate in respect of stability, effects, adverse side effects etc., so a new melanin inhibitor has been desired.

[0007] Cell growth activators are now described hereinafter.

[0008] In aged skin, the activity of skin cells is weakened so that wrinkles and flabby skin are formed. Recently, several studies were carried out with respect to (i) activated skin cells and (ii) the function of the skin itself in a thus activated form, in order to improve skin conditions, and attention has been paid to the development of cell growth activators for activating weakened cells as well as to the incorporation of such cell activators into an agent for external application onto the skin. Conventional materials used for imparting cell-activating activity include alpha-hydroxy acids such as glycolic acids, single-component materials such as hormone, vitamins, photosensitive elements, allantoin etc., and extracted components including animal and plant extracts such as placenta extract, lactobacillus extract, shikon extracts, aloe extract, carrot extract etc. Further, the present inventors also found that there is strong cell-activating activity in distillated residues of solvent extracts etc. from various plants, and filed for a patent (Japanese Patent Application Laid-Open No. 8-284572. October 8, 1996).

[0009] Further, labdanum furanoid diterpenoid (WO 97/45099) are reported as cell differentiation-inducing materials having a labdan structure. However, the majority of conventional materials and extracts having cell-activating activity are unsatisfactory in respect of their effects , so that they have to be applied in a large amount. Also their stability in storage is not satisfactory. Moreover, they may create safety problems because of their stimulating properties or similar natures.

[0010] Anti-bacterial agents are now described hereinafter.

[0011] A large number of microorganisms are present on the skin and many of them are not problematic to a healthy skin, but under bad skin conditions or bad general conditions, these microorganisms invade hair follicles, sweat glands

and damaged sites to act as infection causative agents.

[0012] In addition, there are some microorganisms which cause body odors or dandruff or oxidize secreted lipids to exert adverse effects as causative factors for acne. To kill such microorganisms, many compounds have been used, but many of them are chemically synthesized products, so there has been demand for highly safe anti-bacterial agents derived from natural sources,

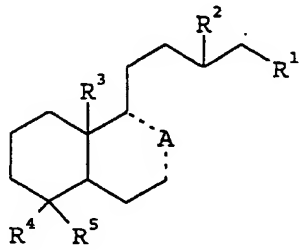
[0013] The anti-bacterial activity of ***Cistus*** absolute as one of extracts from ***Cistus ladaniferus*** L., ***Cistus creticus*** L., ***Cistus monoperiensis*** L., ***Cistus salvifolius*** etc. has already been reported (Nippon Keshohin Gijyutusha Kaishi, 27, 227 (1993)), but its active ingredient is not referred to therein.

SUMMARY OF THE INVENTION

[0014] The object of the present invention is to provide compounds which have a wide variety of biological activities, are derived from natural sources, and are safe and user-friendly.

In particular, the object of the present invention is to provide compounds having an inhibitory activity on production of melanin, a cell-activating activity and an anti-bacterial activity.

[0015] As a result of their eager study to solve this problem, the present inventors found that extracts with hot water, or extracts with ethanol, hexane, etc. of stems, branches, leaves etc. of ***Cistus ladaniferus*** L., ***Cistus creticus*** L., ***Cistus monoperiensis*** L., ***Cistus salvifolius*** etc. have a strong inhibitory activity on production of melanin, cell-activating activity and anti-bacterial activity, that these actions are based on labdanolic acid, and further that labd-7-en-15-oic acid, labd-8(17)-en-15-oic acid, and labd-8-en-15-oic acid obtained by molecular distillation of the above extracts, or of labdanolic acid have a strong inhibitory activity on production of melanin, cell-activating activity and anti-bacterial activity. Further, the present inventors found that salts thereof or methyl and ethyl ester derivatives thereof and reduced derivatives thereof have also the same activity, and as a result of additional examination, the present invention was completed at last. The biologically active substance of the present invention is one or several compounds represented by the following general formula (1):



(1)

wherein R^1 represents $-CH_2OH$, $-COOR^6$, or -COOX, in which X in a group capable of forming a salt and R^6 represents hydrogen or a C_1 to C_3 lower alkyl group; R^2 to R^5 each represent hydrogen or a methyl group; and ---A--- represents $=C(CH_3)-$, $-C(CH_3)=$, $-C(=CH_2)-$, $-CH(CH_3)-$ or $-C(OH)(CH_3)-$.

[0016] In the formula (1), X includes a group capable of forming a salt such as sodium, potassium, ammonium etc., and R^6 includes hydrogen, a methyl group, an ethyl group and a propyl group. In particular X is a physiologically acceptable cation, for instance in therapeutics and/or cosmetics.

[0017] In the present invention, the biologically active substance refers to a substance having one or more activities selected from an inhibitory activity on melanin production, a cell-activating activity and an anti-bacterial activity.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The above compounds are those known in the art and their processes of preparation are also known. For example, labdanolic acid is a component in labdanum gum extracted from ***Cistus ladaniferus*** (J. Chem. Soc., 1956, 4259-4262), and labd-8 (17)-en-15-oic acid (eperuic acid) and labd-8-en-15-oic acid are obtained by chemically treating labdanolic acid (J. Chem. Soc., 1956, 4262-4271). Further, it is reported that eperuic acid is a component in a resin derived from ***Eperua falcata,*** a tree of the ***Leguminosae*** (J. Chem. Soc., 1955, 658-662), and labd-7-en-15-oic acid (cativic acid) is a component in a resin from ***Prioria copaifera*** G., a tree of the ***Leguminosae*** (J. Am. Chem. Soc., Vol. 79, 1201-1205, 1957).

[0019] However, it was not known (i) which biological activity these substances possessed, and (ii) that they exhibit

an inhibitory activity on production of melanin, a cell-activating activity and an anti-bacterial activity.

[0020] Although the plant used for preparing the compounds defined in the present invention is not particularly limited insofar as it is a plant containing said compounds, it is particularly advantageous to employ ***Cistus ladaniferus*** L., ***Cistus creticus*** L., ***Cistus monoperiensis*** L., ***Cistus salvifolius*** plants (of the ***Cistaceae*** family). These are used alone or in combination thereof. The portion of the plant used is not particularly limited, and use is made of leaves, branches, stems, barks etc. These may be used just after harvesting or after drying.

[0021] Preferably, the method of extracting the desired compounds from said plants makes use of one or more solvents selected from the group consisting of water, lower alcohols, petroleum ether and hydrocarbons. The lower alcohols are those containing from 1 to 4 carbon atoms, preferably methanol, ethanol, etc.

[0022] The petroleum ether used may be not only the one known in the art but also a commercial product.

[0023] The hydrocarbon solvents are aliphatic hydrocarbons, alicyclic hydrocarbons and aromatic hydrocarbons which are liquid at ordinary temperatures preferably aliphatic hydrocarbons and aromatic hydrocarbons which are liquid at ordinary temperatures, among which hydrocarbons such as hexane and toluene are particularly preferable.

[0024] Although the operation of extraction differs depending on the plant and solvent used, usually divided pieces of the plant are immersed in the solvent optionally under gentle stirring at a temperature of from room temperature to 50°C.

[0025] Further, the soxhlet extractor known in the art may also be used.

[0026] The time required for extraction is usually of from 3 to 48 hours.

[0027] Alternatively, the method of steam distillation or boiling in hot water after leaves, branches or stems of the plant are disrupted may also be adopted in the present invention. In this case, gum coming to float on water upon steam distillation or hot-water extraction is removed and then separated from insolubles by means of the solvent extraction.

[0028] Further, commercially available products obtained from the above plants by any of the methods described above may be used.

[0029] The crude extract thus obtained contains 25 to 35 % labdanolic acid. This crude extract itself may be used as melanin production inhibitor, cell activator and anti-bacterial agent.

[0030] Hereinafter, a typical method of obtaining the acid or a mixture of the acids from the crude extract or from a commercially available extract is described, but the present invention is not limited to this example.

[0031] The above crude extract or a commercially available extract is subjected to molecular distillation under reduced pressure at 0.1 to 0.5 mm Hg (13.33 to 66.65 Pa) whereby a fraction at 160 to 230°C preferably 180 to 220°C is collected. This fraction contains a mixture of labd-7-en-15-oic acid, labd-8(17)-en-15-oic acid and labd-8-en-15-oic acid.

[0032] As the melanin production inhibitor, cell activator and anti-bacterial agent, this acid mixture may be used as such or if necessary as salts or methyl or ethyl ester derivatives thereof.

[0033] Then, the three acids are separated from this acid mixture.

[0034] Specifically, this acid mixture is dissolved in ethanol, then reacted to form ethyl ester derivatives in the presence of a catalytic amount of sulfuric acid, and subjected to silica gel chromatography on silica gel treated with silver nitrate. The column is washed with hexane and then the ethyl ester derivatives are eluted with 1 % ethyl acetate-hexane. First, labd-8-en-15-oic acid ethyl ester is eluted, then labd-7-en-15-oic acid ethyl ester and finally labd-8(17)-en-15-oic acid ethyl ester are eluted in this order. The solvent is distilled off whereby purified products of the respective ethyl ester derivatives are obtained. Each of the ethyl ester derivatives thus obtained is hydrolyzed to give a free acid, and the free acid is further reacted with diazomethane to give its methyl ester derivative.

[0035] The resulting acids, methyl esters, ethyl esters or mixtures thereof are useful as melanin production inhibitors, cell activators, and anti-bacterial agents.

[0036] Further, these can be incorporated into an agent or composition for external application onto the skin, a bath additive, an oral cavity composition etc. to give a corresponding agent or product having an inhibitory activity on production of melanin, a cell-activating activity and an anti-bacterial activity. Further, the compound (1) of the present invention can be added to prepare an anti-aging agent and an anti-wrinkle agent etc.

[0037] The amount of said melanin production inhibitor, cell activator and anti-bacterial agent incorporated is 0.01 to 10 % by weight, preferably 0.05 to 5 % by weight for the agent for external application onto the skin, 0.1 to 10 % by weight, preferably 0.2 to 5 % by weight for the bath additives, 0.1 to 10 % by weights preferably 0.2 to 5 % by weight for the oral cavity composition, and 0.01 to 5 % by weight, preferably 0.05 to 2 % by weight for the anti-bacterial agent.

[0038] The amount of the compound of the formula (1) is if incorporated into face lotion, milky lotion, cream etc. usually of from 0.05 to 10 % by weight, preferably 0.05 to 2 % by weight.

[0039] The method of incorporation of that compound into a melanin production inhibitor, a cell activator, an anti-bacterial agent etc. is not particularly limited. For example, the compound(s) of the invention may be incorporated after dilution with usual organic solvent used in perfumes, for instance ethylene glycol, propylene glycol and lower alcohols, which are used alone or as a mixture thereof, or after dilution with a mixture of such solvent and a surface active agent,

or after mixing with conventional perfume materials. Alternatively, it may be incorporated as such in the absence of any other material.

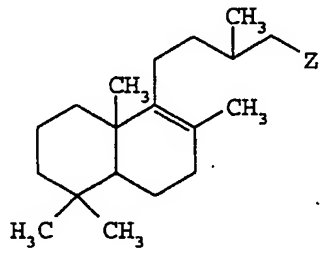
[0040] Further, the melanin production inhibitor, cell activator, anti-bacterial agent etc. of the present invention can contain not only the above essential ingredients but also other ingredients used in agents for external application onto the skin, such as usual cosmetics, quasi drug preparations, pharmaceutical preparations etc. For example, it is possible to incorporate skin whitening agents, cell activators, humectant, antioxidants, oil components, surface active agents, thickeners, inorganic fillers. coloring agents, pH adjusters, preservatives, perfumes, UV absorbers, various skin nutrients etc. depending on the object or the necessity.

[0041] Hereinafter, some of these ingredients are exemplified. As the skin whitening agents, mention can be made of arbutin, kojic acid, ellagic acid, ascorbic acid etc. and various derivatives thereof, as well as extracts from various animals and plants such as placenta extract etc. As the cell activators, mention is made of alpha-hydroxy acids such as glycolic acid etc., hormones, vitamins, various animal and plant extracts. The humectant includes sorbitol, xylitol, glycerin, propylene glycol, sodium pyrrolidonecarboxylate, lactic acid, hyaluronic acid, collagen etc.; the antioxidants include vitamin E, butylhydroxytoluene, butylhydroxyanisol etc.; the oil components include vegetable fats and oils such as liquid paraffin, paraffin, olive oil, coconut oil etc. and animal fats and oils such as tallow, porcine fat, mink oil, squalane etc., and synthetic oils such as methyl polysiloxane, silicone oil, glyceryl triisopalmitate etc.

[0042] The surface active agents include anionic surface active agents such as sodium lauryl sulfate, triethanolamine laurate etc., cationic surface active agents such as cetyltrimethylammonium chloride, benzalkonium chloride etc., nonionic surface active agents such as glyceryl monooleate, sorbitan monostearate, polyoxyethylene hydrogenated castor oil, and sucrose fatty acid ester; the thickeners include carboxymethylcellulose, hydroxyethylcellulose, carboxyvinylpolymers, sodium alginate, carrageenan etc.; the inorganic fillers include talc, sericite, mica, kaolin, zinc white, titanium oxide, magnesium oxide etc.,; the pH adjusters include organic acids, such as citric acid, sodium citrate etc. and salts thereof; the preservatives include urea, parabens such as methyl paraben, ethyl paraben etc., sodium benzoate, ethyl alcohol etc. Further, by adding various UV absorbers, it is also possible to improve the effect of preventing sunburns.

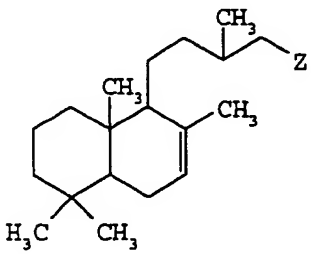
[0043] The preferred biologically active composition according to this invention comprises one substance selected from the group consisting of

(a) labd-8-en-15-oic acid-based compounds of the formula (2a) :



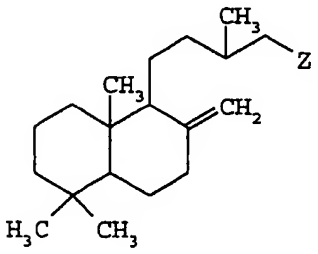
(2a)

(b) labd-7-en-15-oic acid-based compounds of the formula (2b)



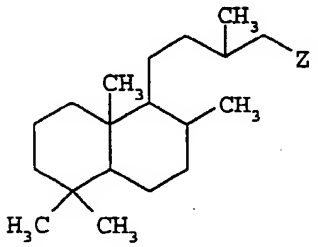
(2b)

(c) labd-8 (17)-en-15-oic acid-based compounds of the formula (2c)



(2c)

(d) labdan-15-oic acid-based compounds of the formula (2d)



(2d)

wherein Z is COOH, COO-(C_1-C_3)alkyl, or CH_2OH

(e) their salts with inorganic bases and their addition salts with organic bases when Z is COOH, and
(f) mixtures thereof.

[0044] The biologically active substance is useful vis-α-vis skin disorders. It is provided here a use of that substance as a melamine production inhibitor, a cell activator and/or an anti-bacterial agent, in the preparation of a skin composition destined to be used in therapy, dermatotherapy and/or cosmetics vis-α-vis skin disorders.

EXAMPLES

[0045] Hereinafter, the present invention is described in more detail by reference to the Examples , which are not intended to limit the present invention.

Example 1

[0046] A commercial absolute (concentrated extract) of labdanum (Givaudan Co., Ltd.) was subjected to molecular distillation. The labdanum absolute(10 g) was subjected to molecular distillation under reduced pressure (0.1 mm Hg, i.e. 13.3 Pa) to collect a fraction (4.3 g) at 180 to 220°C. This fraction contains a mixture of labd-8-en-15-oic acid (Compound 1), labd-7-en-15-oic acid (Compound 4) and labd-8(17)-en-15-oic acid (Compound 7) (this mixture is referred to hereinafter as the acid mixture).

[0047] The acid mixture (1 g) was dissolved in ether (2 ml) and diazomethane was added dropwise thereto to give methyl ester derivatives (0.96 g) (the methyl esters are referred to hereinafter as the methyl ester mixture).

[0048] Similarly, this acid mixture (10 g) was dissolved in ethanol (100 ml) and esterified in the presence of a sulfuric acid catalyst to give ethyl ester derivatives (9.5 g) (the ethyl ester derivatives are referred to hereinafter as the ethyl ester mixture).

Example 2

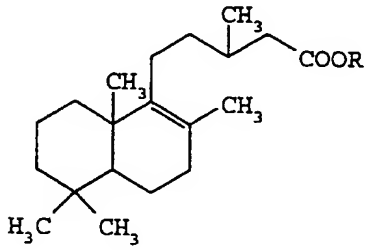
[0049] For separation of these three acids, the ethyl ester mixture was subjected to silica gel chromatography. The

ethyl ester mixture (10 g) was dissolved in hexane (100 ml) and applied to a column packed with silica gel treated with silver nitrate and then eluted with a solvent. The eluting solvent firstly used was hexane and then a mixed solvent of hexane containing 1 % by volume of ethyl acetate. Labd-8-en-15 oic acid ethyl ester was first eluted, then labd-7-en-15-oic acid (cativic acid) ethyl ester and finally labd-8(17)-en-15-oic acid (eperuic acid) ethyl ester were eluted in this order. The eluate containing each of the components was collected and the solvent was distilled off, whereby purified products of the respective ethyl ester derivatives (0.83 g, 0.16 g and 0.63 g in the order of elution) were obtained. Each of the ethyl ester derivatives thus obtained was hydrolyzed to give free acids.

[0050] Further, diazomethane was added dropwise to each free acid, and the solvent was distilled off whereby the methyl ester derivatives were obtained.

Products of the formula 3

[0051]

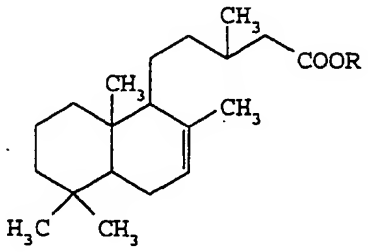


(3)

Compound (1): R=H (labd-8-en-15-oic acid)
Compound (2): R=CH_3
Compound (3): R=C_2H_5

Products of the formula 4

[0052]

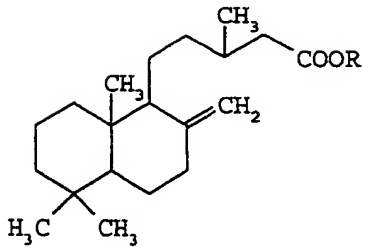


(4)

Compound (4): R=H (labd-7-en-15-oic acid)
Compound (5): R=CH_3
Compound (6): R= C_2H_5

Products of the formula 5

[0053]



(5)

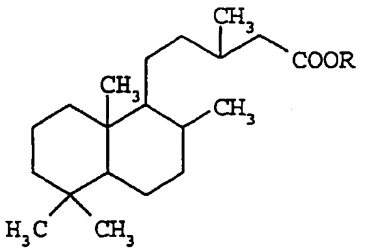
Compound (7): R=H (labd-8(17)-en-15-oic acid)
Compound (8): R=CH_3
Compound (9): R= C_2H_5

Example 3

[0054] The ethyl ester mixture (4.3 g) obtained in Example 1 was dissolved in ethanol (10 ml), and 5 % palladium carbon catalyst (0.2 g) was added thereto for hydrogenation reactions to give Compound 11 (4.1 g). Further, it was hydrolyzed to give Compound 10.

Products of the formula 6

[0055]



(6)

Compound (10): R=H (labdan-15-oic acid)
Compound (11): R= C_2H_5

Example 4

[0056] The ethyl ester mixture (3.2 g) obtained in Example 1 was dissolved in tetrahydrofuran (10 ml) and to this solution a solution of lithium aluminum hydride (0.21 g) in tetrahydrofuran (10 ml) was added dropwise at room temperature to give an alcohol derivative mixture (2.32 g), that is terminus -COOH group of labd-8-en-15-oic acid (Compound 1), labd-7-en-15-oic acid (Compound 4) and labd-8(17)-en-15-oic acid (Compound 7) was replaced with -CH_2OH group. This alcohol derivative mixture may be used directly as a melanin production inhibitor, a cell activator and an anti-bacterial agent.

Test Example 1

Melanin formation prevention test

[0057] B16 melanoma cells were suspended (10,000 cells/ml) in DMEM (Dulbecco's modified eagle medium) containing 10 % FBS (fetal bovine serum) and 8 ml of the suspension was added to a cell culture bottle with a 25 cm^2 bottom surface area, and cultured at 37°C in the presence of 5 % carbon dioxide for 3 days. After 3 days of culture, the old medium was exchanged with 8 ml of fresh medium. 40 µl of a sample solution containing a compound of the invention, which had been dissolved in ethanol to give a final concentration shown in the table, was further added thereto. As the control, ethanol only was added. After the medium change, the cells were further cultured for 3 days under the same conditions. After culture was finished, the medium was removed, and the cells were recovered by treatment with trypsin (DIFCO) and suspended in 4 ml of phosphate buffered saline (PBS) and a predetermined amount (1/40) of this suspension was used to measure the number of cells in a Coultar counter (Sysmex Co.) to determine the degree of cell proliferation.

[0058] The degree of cell proliferation was determined according to the following equation : Degree of cell proliferation (%) = (number of cells in sample)/(number of cells in control) x 100

[0059] The remainder of the cell suspension was centrifuged and then washed with 5 % trichloroacetic acid, then with ethanol/ethyl ether (3 : 1 by volume), and with ethyl ether and the cells were dried, and 2N NaOH was added thereto to dissolve (melanin in) the cells under heating at 70°C and the measure of the optical density (OD) was carried out at 420 nm. Using a calibration curve of synthetic melanin, the amount of melanin/million cells was determined from the OD and the degree of inhibition of melanin production was determined according to the following equation: Degree of inhibition of melanin (%) = (amount of melanin in control - amount of melanin in sample)/(amount of melanin in control) x 100

[0060] A degree of inhibition of melanin of 60 % or more and a degree of cell proliferation of 70 % or more are indicative of superior safety and superior inhibitory activity on production of melanin, so that highly practical usage can be envisaged.

Table 1

Degree of inhibition of melanin production			
Sample	Concentration (ppm)	Degree of melanin inhibition	Degree of cell proliferation
Crude extract	6.3	75%	110%
Acid mixture	6.3	77%	143%
Methyl ester mixture	6.3	80%	122%
Ethyl ester mixture	6.3	75%	122%
Compound 1	3.1	80%	109%
Compound 3	6.3	74%	115%
Compound 4	6.3	87%	109%
Compound 6	6.3	74%	109%
Compound 7	6.3	79%	111%
Compound 9	6.3	61%	103%
Compound 10	6.3	83%	97%
Compound 11	6.3	75%	107%
Kojic acid	200.0	34%	94%
Arbutin	6.3	74%	100%
Ellagic acid	3.1	68%	98%

Test Example 2

Combination test with existing tyrosinase inhibitors

[0061] Using the method of Test Example 1, arbutin, kojic acid, and ellagic acid, which were known to have an inhibitory activity on tyrosinase, were mixed with the compound of the present invention and examined for the degree of inhibition of melanin production in B16 melanoma cells as well as for the degree of proliferation of the cells, to determine the effect of their combined use.

Table 2

Effect of combined use with arbutin			
Sample	Concentration (ppm)	Degree of melanin inhibition	Degree of cell proliferation
Acid mixture (A)	0.4	9%	103%
Compound 1 (B)	0.4	22%	115%
Compound 4 (C)	0.4	28%	115%
Arbutin (E)	0.8	22%	89%
(A) + (E)	0.4 + 0.8	52%	128%
(B) + (E)	0.4 + 0.8	54%	132%
(C) + (E)	0.4 + 0.8	56%	127%

Table 3

Effect of combined use with kojic acid			
Sample	Concentration (ppm)	Degree of melanin inhibition	Degree of cell proliferation
Acid mixture (A)	0.4	37%	114%
Compound 1 (B)	0.4	46%	122%
Compound 4 (C)	0.4	51%	119%
Compound 7 (D)	0.4	34%	130%
Kojic acid (E)	200	34%	94%
(A)+(E)	0.4 + 200	55%	96%
(B)+(E)	0.4 + 200	43%	96%
(C)+(E)	0.4 + 200	71%	96%
(D)+(E)	0.4 + 200	54%	113%

Table 4

Effect of combined use with ellagic acid			
Sample	Concentration (ppm)	Degree of melanin inhibition	Degree of cell proliferation
Acid mixture (A)	0.4	17%	106%
Compound 7 (B)	0.4	30%	111%
Ellagic acid (C)	1.6	14%	98%
(A)+(C)	0.4 + 1.6	31%	111%
(B)+(C)	0.4 + 1.6	31%	100%

[0062] As can be seen from Tables 2, 3 and 4, when arbutin, kojic acid, and ellagic acid were used with a compound of the present invention, they showed an higher inhibitory activity on melanin production than their use alone. The degree of cell proliferation was also higher than when these tyrosinase inhibitors were used alone. This indicates that these known materials used in combination with the compounds of the present invention bring about a higher effect as skin whitening agent.

Test Example 3

Effect of diminishing UV ray-induced pigment stains in guinea pigs

[0063] Hair was carefully removed from the back of 5 brown guinea pigs, and a shielding plate provided with four 2.5 cm X 2.5 cm openings was attached to the portion from which hair had been removed, followed by irradiating it 3 times every second day with UV_B rays at an intensity of 450 mj/cm^2. Immediately after the irradiation of UV_B rays, 70 µl sample was applied onto the irradiated site once per day for 35 days, and the amount of pigment diminished by this treatment was examined on the days shown in Table 5. The sample used was an ethanol solution containing 1 % acid mixture obtained in Example 4 as the compound of the invention, and ethanol only was applied as the control.

[0064] For evaluation of the activity, each application site was measured by a colorimeter (CR200b, Minolta Co Ltd.). ΔLx value was calculated by subtracting the L value of the portion before the sample application started from the L value after the sample application (value changing with time), and then ΔΔL value was determined by subtracting ΔLo value (i. e. the value similarly determined for the portion to which ethanol was applied) from the ΔLx value.

[0065] The ΔΔL value can be determined according to the following equation:

$$\Delta\Delta L = (Lo - Lx) - (L'o - L'x)$$

Lo: L value of the test site (site to which the sample was applied) before sample application
Lx : L value of the test site (site to which the sample was applied) on Day x after sample application
L'o : L value of the control site (site to which ethanol was applied) before ethanol application
L'x : L value of the control site (site to which ethanol was applied) on Day x after ethanol application

[0066] The same experiment was conducted using an ethanol solution containing 7 % kojic acid. The results are shown in Table 5.

Table 5

Change in ΔΔL value				
Sample	Concentration	ΔΔL value		
		Day 14	Day 28	Day 35
Acid mixture	1%	0.30	2.25	2.15
Kojic acid	7%	1.32	1.52	1.40

[0067] As can be seen from Table 5, the acid mixture as one of the compounds of the present invention has an evident action of diminishing pigment stains as compared with the control. The activity was stronger than that or kojic acid after Day 28. When observed with the passage of time, kojic acid was found to indicate the activity at an earlier stage until Day 14.

Test Example 4

Mushroom tyrosinase inhibition test

[0068] Commercial mushroom-derived tyrosinase (Sigma) was used to examine inhibitory activity on tyrosinase. 0.2 ml sample solution was added to 2.3 ml phosphate buffer so as to give a final concentration shown in Table 6, then 0.1 ml of a tyrosinase solution (1000 U/ml) was added thereto, and further 0.4 ml of L-tyrosine solution (0.3 mg/ml) was added thereto as the substrate, and the mixture was kept at 37°C for 30 minutes. After reaction, the absorbance (OD) at a wavelength of 490 nm was measured and the degree of inhibition of tyrosinase reaction was determined according to the following equation. For comparison, arbutin and kojic acid known as tyrosinase inhibitors were also examined.

[0069] Degree of inhibition (%) = (1- ((sample OD - blank OD)/(Sample-free OD - blank OD))) x 100

[0070] Sample: The buffer, the enzyme solution, the substrate solution and the sample solution

[0071] Sample-free: The buffer, the enzyme solution and the substrate solution

[0072] Blank: The buffer and the enzyme solution

Table 6

Degree of inhibition of mushroom tyrosinase		
Sample	Concentration (ppm)	Degree of inhibition
Acid mixture	25	0%
Compound 1	25	0%
Compound 4	25	0%
Compound 7	25	0%
Arbutin	100	29%
Kojic acid	25	67%

[0073] As shown in Table 6, arbutin and kojic acid known as tyrosinase inhibitors inhibited mushroom tyrosinase, while the compounds of the present invention as melanin production inhibitors did not show any inhibitory activity, thus suggesting the possibility that their activity does not lie in the inhibition of tyrosinase.

Test Example 5

Test of tyrosinase inhibition in B16 melanoma cells

[0074] B16 melanoma cells were cultured for 3 days in DMEM containing 10% FBS at 37°C in the presence of 5 % carbon dioxide. The cells proliferated after culture were recovered by treatment with trypsin, then suspended in PBS containing 0.1 % Triton X100 at a density of ten million cells/ml and disrupted by sonication. The product obtained was centrifuged at 11,000 G for 20 minutes, and the resulting supernatant was used as a crude enzyme solution. 0.2 ml sample solution was prepared in a phosphate buffer so as to give a final concentration shown in Table 7. Thereto was added 0.2 ml of crude enzyme solution. The mixture was preliminarily kept at 37°C for 5 minutes, and 0.2 ml L-DOPA solution (0.5 mg/ml) was added as the substrate, and the mixture was kept at 37°C for 3 hours. After 3 hours, the absorbance (OD) at a wavelength of 490 nm was measured, and the degree of inhibition of tyrosinase was determined according to the equation shown in Test Example 4. For comparisons arbutin and kojic acid known as tyrosinase inhibitors were also examined.

Table 7

Degree of inhibition of tyrosinase in B16 melanoma cells		
Sample	Concentration (ppm)	Degree of inhibition
Acid mixture	25	0%
Compound 1	25	0%
Compound 4	25	0%
Compound 7	25	0%
Arbutin	100	35%
Kojic acid	25	62%

[0075] As shown in Table 7, arbutin and kojic acid known as tyrosinase inhibitors inhibited tyrosinase in B16 melanoma cells, while the compounds of the present invention as melanin production inhibitor did not exhibit any inhibitory activity.

Test Example 6

Test of cell activating activity

[0076] Human-derived normal skin fibroblasts (NBIRGB: Institute of Physical and Chemical Research) were suspended into DMEM containing 10 % FBS to give a concentration of 20,000 cells/ml, and 5 ml each of this cellular suspension was introduced into each 25 cm^2 bottles, cultured at 37°C in the presence of 5 % carbon dioxide for 24 hours. To each bottles, was added 10 μl (0,2 %) of the ethanol solution containing the compound to give the final concentration shown in Table 8. The cells were cultured for additional 3 days. After 3 days the old medium was discarded and 5 ml fresh medium was added, and the sample was further added thereto. After this exchange of the medium, the cells were further cultured for another 3 days. Then cells were removed with trypsin and the number of cells in each bottle was counted with a coultar counter.

[0077] Simultaneously, as the control, only ethanol was added and the NB1RGB cells were cultured and the number of cells were counted in the same manner.

[0078] The number of cells in each bottle to which the sample had been added was determined as a relative value to the number (as 100) of cells in the control after cultured, and the results are shown in Table 8. As the comparative example, glycolic acid known to have cell activating activity was examined and shown in the table.

Table 8

Degree of cell proliferation of fibroblasts		
Sample	Concentration (ppm)	Degree of cell proliferation
Crude mixture	8.0	130%
Acid mixture	8.0	130%
Methyl ester mixture	4.0	126%
Ethyl ester mixture	4.0	110%
Compound 3	8.0	134%
Compound 6	8.0	134%
Compound 7	8.0	128%
Compound 9	8.0	127%
Compound 10	8.0	126%
Compound 11	8.0	104%
Alcohol derivative mixture	4.0	138%
Glycolic acid	4.0	120%

[0079] As shown in Table 8, the compounds of the present invention showed the strong activity of activating proliferation of NB1RGB fibroblasts.

Test Example 7

Anti-bacterial activity test

[0080] A test was conducted using the 8 aerobic microorganisms and 2 anaerobic microorganisms shown in Table 9 below. The aerobic microorganisms were examined in an agar medium dilution method and the anaerobic microorganisms were examined in a liquid medium dilution method under anaerobic culture conditions.

Agar medium dilution method

[0081] Muller Hinton agar medium (DIFCO) was heated and dissolved and 10 ml of the medium was introduced into each test tube, sterilized and used. The sample was prepared by dissolving in ethanol and the resulting ethanol solution was diluted 2-fold serially with ethanol, and 100 µl of sample solution was added to 10 ml of each dissolved agar medium, then stirred, introduced into a Petri dish of 9 cm in diameter, and solidified at room temperature. Each test microorganism in a well grown slant was inoculated via one loop of platinum into 10 ml Muller Hinton broth (DIFCO) and cultured with shaking at 27°C for 24 hours and used as a microbial fluid. This fluid was diluted at 10^8 CFU (Colony forming unit)/ml, and 5 pl of the diluted fluid was inoculated onto the agar and cultured at 37°C overnight. Inoculation of the microorganism was conducted using microplanter MIT-P for 7 microorganisms in test tubes Sakuma Seisakusho K. K.).

[0082] Measurement of MIC (minimum inhibitory concentration) was conducted by comparison with the control where the test microorganism was inoculated and grown onto a Muller Hinton agar plate containing 100 µl ethanol, and the concentration at which the microorganism did not grow was regarded as MIC.

Liquid Medium dilution method

[0083] 10 ml GAM broth (Nissui) was introduced into each test tube equipped with a cap and sterilized. 100 µl of the sample solution prepared for agar medium dilution method was added to each test tube and lightly stirred, and 100 µl test microbial solution (10^7 CFU/ml) was added thereto, then capped and incubated at 37°C. The test microbial solution was prepared by introducing 100 µl stock microbial solution into 10 ml GAM broth in a test tube then capping the tube and fermented it at 37°C overnight.

[0084] Measurement of MIC was conducted by comparison with the control to which 100 µl ethanol was added, and the concentration at which the microorganism did not grow was regarded as MIC.

Table 9

Test Microorganisms	
Strains	Test Microorganisme Code
Aerobic microorganisms :	
Staphylococcus epidermidis JCM 2414	Se-1
Staphylococcus epidermidis var. H-6	Se-2
Corynebacterium minutissimum ATCC 23348	Cm-1
Corynebacterium xerosis JCM 1324	Cx-2
Malassezia furfur IFO 0656	Mf-1
Staphylococcus aureus IFO 12732	Sa-3
Bacillus subtilis PCI 219 IFO 3134	Bs-1
Anaerobic microorganisms :	
Propionibacterium acnes ATCC 12818	Pa-1
Streptococcus mutans JCM 5175	Su-1

Table 10

Results of the anti-bacterial test (MIC: ppm)					
	Samples				
Test Microorganism code	Acid mixture	Compound 1	Compound 4	Compound 7	Compound 10
Se-1	12.5	6.3	12.5	6.3	6.3
Se-2	12.5	6.3	12.5	6.3	6.3
Cm-1	12.5	6.3	6.3	12.5	6.3
Cx-2	12.5	12.5	12.5	12.5	6.3
Mf-1	6.3	6.3	6.3	6.3	6.3
Sa-3	12.5	12.5	12.5	12.5	6.3
Bs-1	12.5	6.3	12.5	12.5	6.3
Pa-1	NT	12.5	NT	NT	12.5
Su-1	NT	6.3	NT	NT	6.3

[0085] In table 10, NT means that the test was not conducted.

[0086] As shown in Table 10, the free acids as the compounds of the invention exhibited strong activity toward causative bacteria for body odors (Se-1, Se-2), for dandruff (Mf-1), for acne (Pa-1), for caries (Su-1) etc.

Example 5

[0087] The melanin production inhibitor of the present invention was used to prepare face lotion, milky lotion, cream, pack, bath additive and cream foundation respectively.

(1)Face lotion (see Table 11)

[0088]

Table 11

Ingredients	Incorporation amount (% by weight)
Conc. Glycerin	3.0
1,3-Butylene glycol	2.0
Polyoxyethylene sorbitan monolaurate	1.0
Ethanol	5.0
Perfume	suitable amount

Table 11 (continued)

Ingredients	Incorporation amount (% by weight)
Acid mixture (Example 1)	1.0
Preservative	suitable amount
Purified water	Adjusted to 100%

(2) ***Milky lotion*** (see Table 12)

[0089]

Table 12

Ingredients	Incorporation amount (% by weight)
Squalane	5.0
Vaseline	2.0
Beeswax	0.5
Sorbitan sesquioleate	0.8
Polyoxyethylene oleyl ether (20 E.O.)	1.2
Compound 1 (Example 2)	0.5
Perfume	suitable amount
Preservative	suitable amount
Humectant (propylene glycol)	5.0
Ethanol	5.0
Viscous material	20.0
(1.0 % aq. Carboxyvinyl polymer)	0.1
Alkali (potassium hydroxide)	
Purified water	Adjusted to 100%

(3) ***Cream*** (see Table 13)

[0090]

Table 13

Ingredients	Incorporation amount (% by weight)
Squalane	5.0
Vaseline	2.0
Beeswax	0.5
Sorbitan sesquioleate	0.8
Polyoxyethylene oleyl ether (20 E.O.)	1.2
Perfume	suitable amount
Ethyl ester mixture (Example 1)	1.0
Preservative	suitable amount
Humectant (propylene glycol)	5.0
Ethanol	5.0
Viscous material	20.0
(1.0 % aq. Carboxyvinyl polymer)	0.1
Alkali (potassium hydroxide)	
Purified water	Adjusted to 100%

(4) ***Pack*** (see Table 14)

[0091]

Table 14

Ingredients	Incorporation amount (% by weight)
Polyvinyl alcohol	15.0
Carboxymethylcellulose sodium	5.0
Propylene glycol	3.0
Ethanol	10.0
Perfume composition	suitable amount
Compound 10 (Example 3)	1.0
Preservative and antioxidant	suitable amount
Purified water	Adjusted to 100%

(5) ***Bath additive*** (granular type) (see Table 15)

[0092]

Table 15

Ingredients	Incorporation amount (% by weight)
Sodium sulfate	45
Sodium bicarbonate	51.5
Borax	2
Carboxymethylcellulose sodium	1
Pigments	Suitable amount
Perfume	Suitable amount
Compound 4 (Example 2)	0.5

(6) ***Cream foundation*** (see Table 16)

[0093]

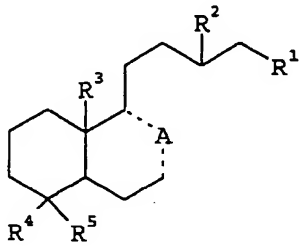
Table 16

Ingredients	Incorporation amount (% by weight)
Stearic acid	5.0
Lipophilic glycerin monostearate	2.5
Cetostearyl alcohol	1.0
Propylene glycol monolaurate	3.0
Liquid paraffin	7.0
Isopropyl myristate	8.0
Butyl paraoxybenzoate	suitable amount
Triethanolamine	1.2
Sorbitol	3.0
Methyl p-oxybenzoate	suitable amount
Titanium oxide	8.0
Koalin	5.0
Talk	2.0
Bentonite	1.0
Coloring pigment	suitable amount
Acid mixture (Example 1)	1.0
Purified water	Adjusted to 100%

[0094] According to the present invention, it was revealed that purified products of extracts from specific plants have excellent inhibitory activity on production of melanin, cell-activating activity, and anti-bacterial activity. The melanin inhibitor consisting of this purified product is excellent not only as a skin whitening agent for preventing the formation of stains and freckles, as well as deposition of pigment in the skin after sunburn and for improving appearances, but also superior in safety and product stability. Further, this purified product can be used as an agent for external application onto the skin, which is effective for prevention and treatment of body odors, dandruff, wounds etc. by activating skin cells themselves and activating the functions of the skin itself to improve skin conditions. These biologically active substances can be incorporated into base cosmetics such as cream, lotion, milky lotion, pack etc., make-up cosmetics such as foundation etc. bath additive, agent for external application onto the skin, oral cavity composition etc.

Claims

1. A biologically active composition comprising one or several compounds represented by the following general formula (1):



(1)

wherein R^1 represents CH_2OH, $-COOR^6$ or -COOX, in which X is a group capable of forming a salt and R^6 represents hydrogen or a C_1 to C_3 lower alkyl group; R^2 to R^5 each represent hydrogen or a methyl group; and ---A--- represents $=C(CH_3)-$, $-C(CH_3)=$, $-C(=CH_2)-$, $-CH(CH_3)-$ or $-C(OH)(CH_3)-$.

2. A biologically active composition according to claim 1 wherein the compounds represented by the general formula (1) are compounds prepared from extracts of ***Cistus ladaniferus*** L., ***Cistus creticus*** L., ***Cistus monoperiensis*** L., ***Cistus salvifolius*** plants ***(Cistaceae)***

3. A melanin production inhibitor composition comprising one or several compounds described in claim 1.

4. A cell activator composition comprising one or several compounds described in claim 1.

5. An anti-bacterial composition comprising one or several compounds described in claim 1.

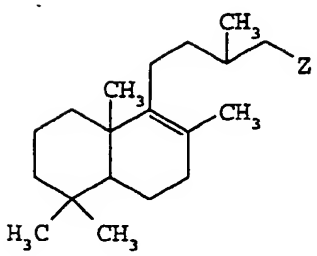
6. A composition for external application onto the skin comprising one or several compounds described in claim 1 or 2.

7. An oral cavity composition comprising one or several compounds described in claim 1 or 2.

8. A bath additive comprising one or several compounds described in claim 1 or 2.

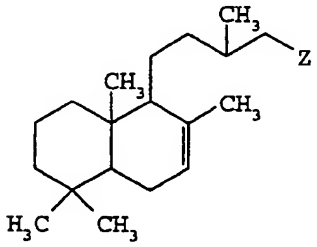
9. A biologically active composition according to claim 1 or 2, comprising one substance selected from the group consisting of

(a) labd-8-en-15-oic acid-based compounds of the formula (2a) :



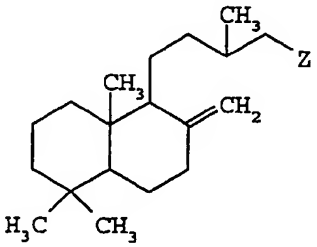
(2a)

(b) labd-7-en-15-oic acid-based compounds of the formula (2b)



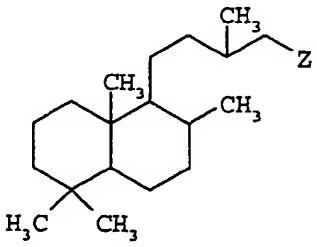
(2b)

(c) labd-8 (17)-en-15-oic acid-based compounds of the formula (2c)



(2c)

(d) labdan-15-oic acid-based compounds of the formula (2d)



(2d)

wherein Z is COOH, COO-(C_1-C_3)alkyl, or CH_2OH,

(e) their salts with inorganic bases and their addition salts with organic bases when Z is COOH, and

(f) mixtures thereof.

10. A use of one or several compounds of the formula (1) according to claim 1 or 2 as a biological active agent.

11. A use of a compound selected from the group consisting of labd-8-en-15-oic acid-based compounds, labd-7-en-15-oic acid-based compounds, labd-8(17)-en-15-oic acid-based compounds and mixtures thereof according to claim 9, as a melanin production inhibitor.

12. A use of a compound selected from the group consisting of labd-8-en-15-oic acid-based compounds, labd-7-en-15-oic acid-based compounds, labd-8(17)-en-15-oic acid-based compounds and mixtures thereof according to claim 9, as a cell activator.

13. A use of a compound selected from the group consisting of labd-8-en-15-oic acid-based compounds, labd-7-en-15-oic acid-based compounds, labd-8(17)-en-15-oic acid-based compounds and mixtures thereof according to claim 9, as an anti-bacterial agent.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 99 40 0933

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	PATENT ABSTRACTS OF JAPAN vol. 0, no. 0 & JP 07 206654 A (OCHIAI MICHIO), 8 August 1995 (1995-08-08) * abstract *	1,3,6, 9-11	A61K31/19 A61K31/215 A61K31/045 A61K7/48 A61K7/16
Y		2	
X	CHEMICAL ABSTRACTS, vol. 88, no. 17, 24 April 1978 (1978-04-24) Columbus, Ohio, US; abstract no. 116228, CUTLER, HORACE G. ET AL: "Plant growth inhibiting properties of diterpenes from tobacco" XP002117657 * abstract * & PLANT CELL PHYSIOL., 1977, 18, 711-14, * pages 712 and 713 labdanediol *	1	
Y	CHEMICAL ABSTRACTS, vol. 76, no. 17, 24 April 1972 (1972-04-24) Columbus, Ohio, US; abstract no. 096966, TABACIK, CHRISTIANE ET AL: "Chemotaxonomic study in the genus Cistus" XP002117658 * page 3093, last paragraph * * page 3094, paragraph 1 * * page 3095 compounds VII, XV, XVI * & PHYTOCHEMISTRY, 1971, 10, 3093-106,	2	TECHNICAL FIELDS SEARCHED (Int.Cl.6) A61K
Y	DE PASCUAL T J ET AL: "STUDY OF THE GUM RESIN OF #CISTUS#-LADANIFERUS PART 4 #LADENIC#-ACID #LABD#-8-20-#EN#-15-#OIC#-ACID" AN QUIM, XP002117655 * page 719 formulas I to IV *	2	

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14 October 1999	Gac, G

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
O : non-written disclosure
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

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EUROPEAN SEARCH REPORT

Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	DE PASCUAL ET AL.: "Reactivity of methyl labdanolate with lead tetraacetate" TETRAHEDRON, vol. 42, no. 16, 1986, pages 4563-4576, XP002117656 * page 4565 compounds 1-6 *	1,2,9,10	
A	CHEMICAL ABSTRACTS, vol. 125, no. 17, 21 October 1996 (1996-10-21) Columbus, Ohio, US; abstract no. 222205, URONES, JULIO G. ET AL: "Labdanolic acid: synthetic precursor of tricyclic diterpenes" XP002117659 * abstract * & NAT. PROD. LETT., 1995, 6, 285-290, * page 286 compounds 1a, 1b, 2a, 2b and 2c * * page 287 compounds 1a and 2b *	1,2,9,10	
A	CHEMICAL ABSTRACTS, vol. 97, no. 13, 27 September 1982 (1982-09-27) Columbus, Ohio, US; abstract no. 107000, DE PASCUAL TERESA, J. ET AL: "Labdane diterpenoids from Cistus ladaniferus" XP002118871 * abstract * & PHYTOCHEMISTRY, 1982, 21, 899-901, * page 900 compounds 7 and 8 *	1,2,9	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14 October 1999	Gac, G

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L : document cited for other reasons

& : member of the same patent family, corresponding document

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EUROPEAN SEARCH REPORT

Application Number
EP 99 40 0933

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A,D	COCKER ET AL.: "The chemistry of gum labdanum. Part I and Part II" J. CHEM. SOC.,1956, pages 4259-4271, XP002118866 * page 4259 first paragraph * * page 4263 compounds I-VI, XII and page 4265 compounds XVIII and XX *	1,2,9,10	
A	DEMETZOS C ET AL: "CYTOTOXIC ACTIVITY OF LABDANE TYPE DITERPENES ISOLATED FROM THE LEAVES OF #CISTUS#-INCANUS-SSP-CRETICUS L. HEYWOOD" THIRD INTERNATIONAL CONFERENCE OF ANTICANCER RESEARCH, MARATHON, GREECE, OCTOBER 16-20, 1990. ANTICANCER RES, XP002118867 * abstract 141 *	1,4,9, 10,12	
A	BARRERO: "Synthesis of biologically active drimanes and homodrimanes from (-)sclareol" TETRAHEDRON, vol. 51, no. 27, 1995, pages 7435-7450, XP002118868 * pages 7436-7439, page 7749 *	1,5,9, 10,13	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
A	URZUA ET AL.: "Comparative antimicrobial study of the resinous exudates of some Chilean Haplopappus (Asteraceae)" J. ETHNOPHARMACOL., vol. 45, 1995, pages 71-74, XP002118869 * the whole document *	1,5,9, 10,13	
A	PATENT ABSTRACTS OF JAPAN vol. 0, no. 0 & JP 07 025754 A (NOZAWA SUSUMU), 27 January 1995 (1995-01-27) * abstract *	1,3,6, 9-11	

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14 October 1999	Gac, G

CATEGORY OF CITED DOCUMENTS

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EUROPEAN SEARCH REPORT

Application Number
EP 99 40 0933

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
P,X	URZUA A ET AL: "Antimicrobial study of the resinous exudate and of diterpenoids isolated from Eupatorium salvia (Asteraceae)." J ETHNOPHARMACOL, OCT 1998, 62 (3) P251-4, XP002118870 IRELAND * the whole document *	1,5,9, 10,13	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14 October 1999	Gac, G

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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14-10-1999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 07206654 A	08-08-1995	NONE	
JP 07025754 A	27-01-1995	NONE	

EPO FORM P0459

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